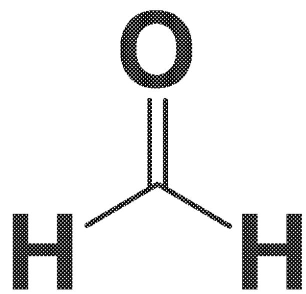
Critical Issues for Formaldehyde Cancer Risk Assessment

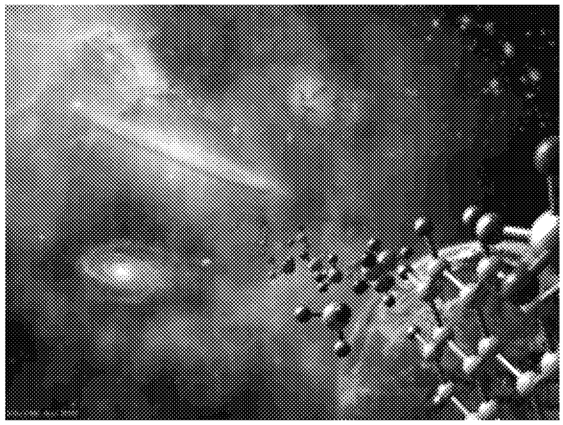


James Swenberg, D.V.M., Ph.D., DACVP

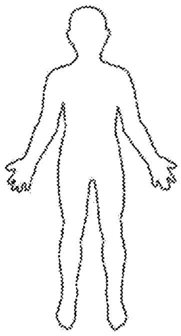
University of North Carolina

Chapel Hill, NC

Formaldehyde is One of the Oldest Chemicals in the World



Formaldehyde was
Part of the Origin of Life

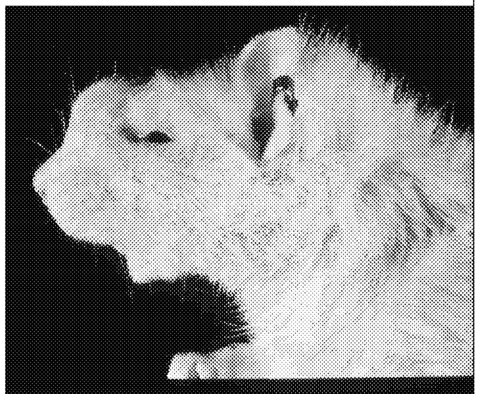


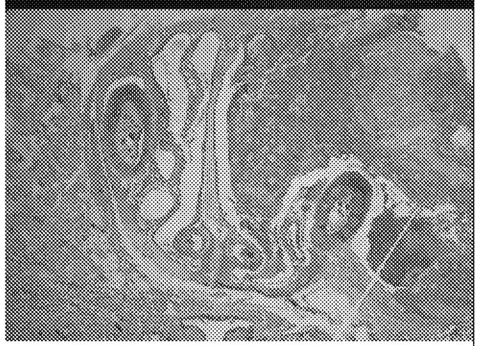
Sources of
Endogenous
Formaldehyde

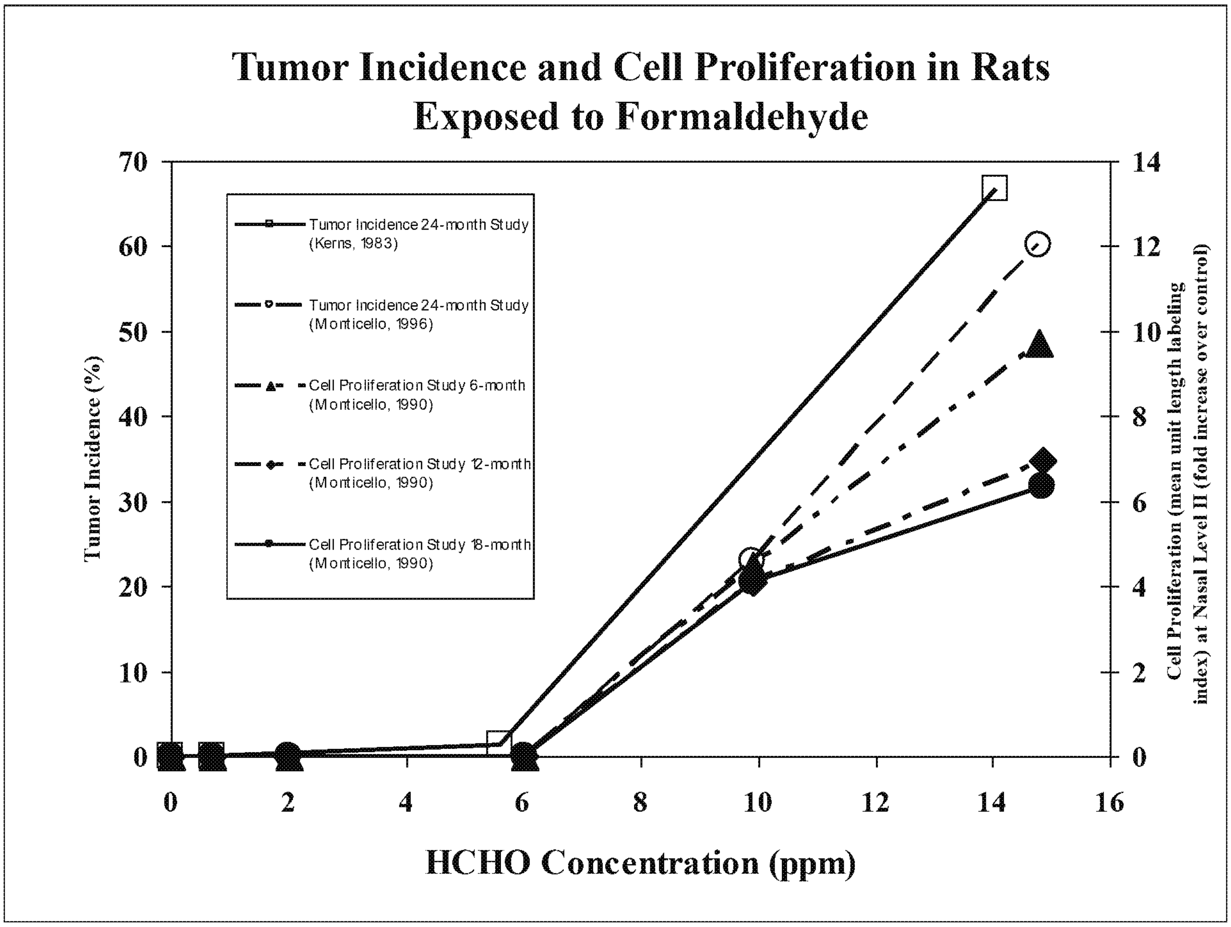
- One-carbon pool
- Methanol metabolism
- Amino Acid metabolism
- Lipid Peroxidation
- P450 dependent demethylation (O-, N-, S-methyl)

Carcinogenesis Bioassays

- CIIT/Battelle studies in rats and mice
  - 12 month sacrifice/interim report
  - 18 month data published in Cancer Research (Swenberg ,et al 1980)
  - Final report and Cancer Research paper on the study (Kerns, et al. 1983)
- CIIT expanded the exposure range and mechanistic designs in a second bioassay published in Cancer Research (Monticello, et al, 1996)
- Subsequent cancer bioassays
  - Inhalation studies
  - Oral studies





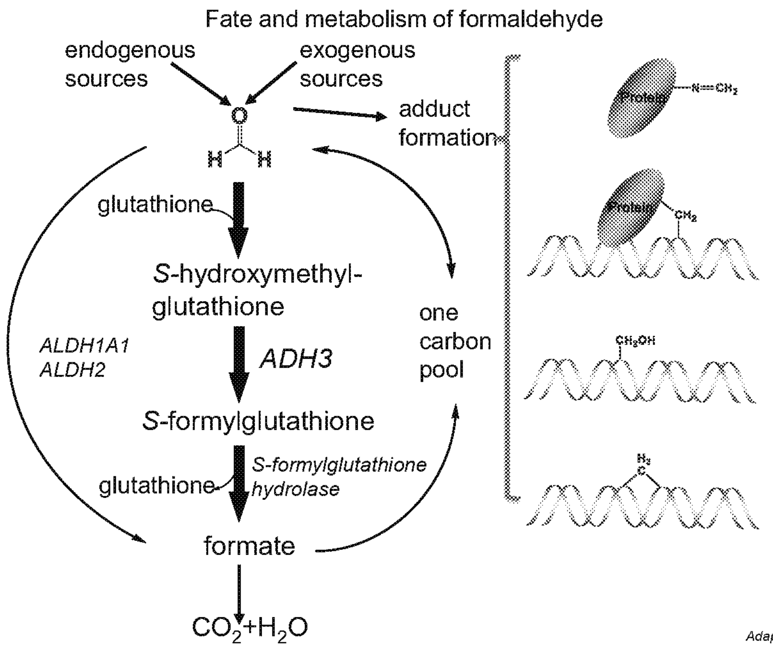

Tumor Incidence and Cell Proliferation in Rats
Exposed to Formaldehyde
Tumor Incidence 24-month Study (Kerns, 1983)
Tumor Incidence 24-month Study (Monticello, 1996)
Cell Proliferation Study 6-month (Monticello, 1990)
Cell Proliferation Study 12-month (Monticello, 1990)
Cell Proliferation Study 18-month (Monticello, 1990)
Tumor Incidence (%)
Cell Proliferation (mean unit length labeling index) at Nasal Level II (fold increase over control)
HCHO Concentration (ppm)
0
10
20
30
40
50
60
70
2
4
6
8
10
12
14
16

Early Mode of Action Studies

- Cytotoxicity and cell proliferation studies
  - Cell proliferation is a key factor in converting DNA damage to mutations
- Minute volume studies comparing rats and mice
- DNA-protein cross-link quantitation
  - Careful assays based on physical chemistry were conducted in rats and primates
  - Demonstrated nonlinear exposure relationships
  - Did not find any accumulation in multiple day exposures

Recent Molecular Mode of Action Studies

Formaldehyde is very reactive with proteins and DNA, leading to diverse protein adducts and DNA damage.



Adapted for IARC monograph 88

Formaldehyde Specific DNA Adducts

$^{13}CD_2O$ Exposure

↓

Tissue Collection

↓

DNA Isolation

↓

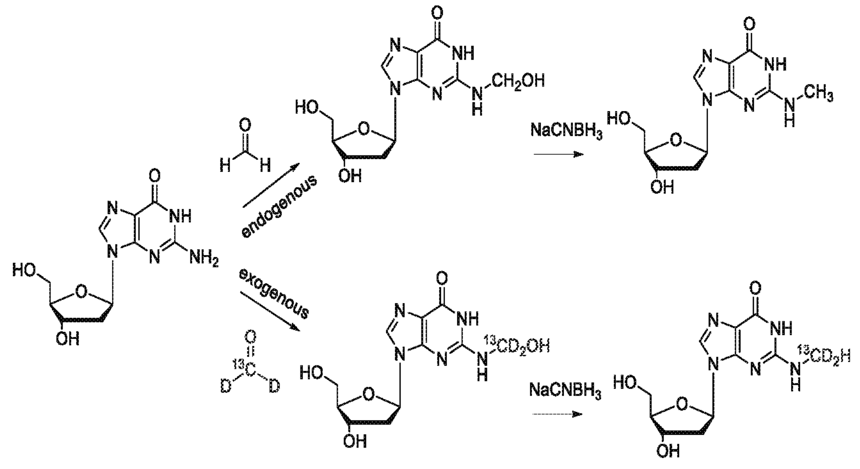
Reduction with $NaCNBH_3$

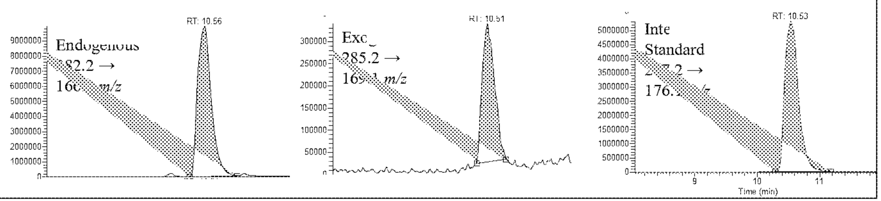
↓

Digestion and HPLC Fractionation

↓

Nano-LC-MS/MS

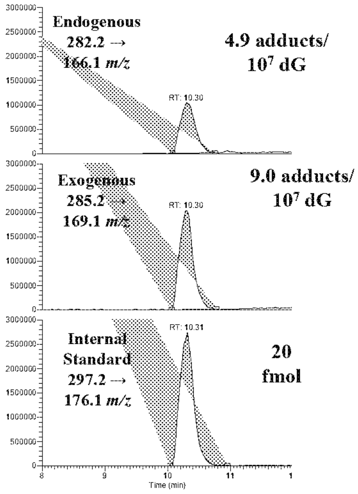




Formaldehyde-induced N^2-hydroxymethyl-dG adducts in rats exposed to 10 ppm Formaldehyde for 1 or 5 days

Exposure Period	Tissues	Exogenous adducts/10^7 dG	Endogenous adducts/10^7 dG
1 day	Nose	1.28 ± 0.49	2.63 ± 0.73
	Lung	nd	2.39 ± 0.16
	Liver	nd	2.66 ± 0.53
	Spleen	nd	2.35 ± 0.31
	Bone Marrow	nd	1.05 ± 0.14
	Thymus	nd	2.19 ± 0.36
	Blood	nd	1.28 ± 0.38
5 day	Nose	2.43 ± 0.78	2.84 ± 1.13
	Lung	nd	2.61 ± 0.35
	Liver	nd	3.24 ± 0.42
	Spleen	nd	2.35 ± 0.59
	Bone Marrow	nd	1.17 ± 0.35
	Thymus	nd	1.99 ± 0.30
	Blood	nd	1.10 ± 0.28

Dosimetry of N^2-hydroxymethyl-dG Adducts in Nasal Epithelium of Rats

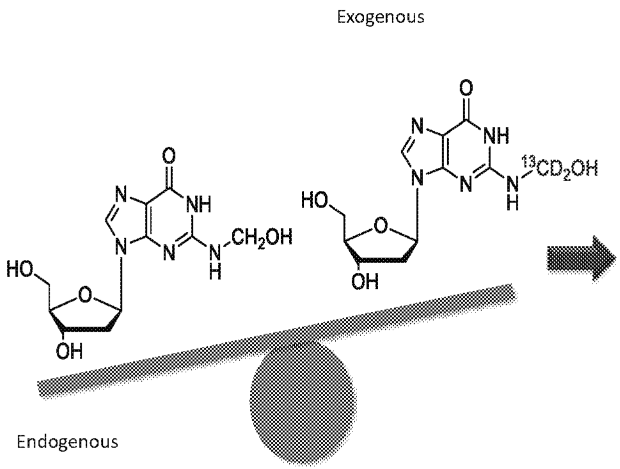


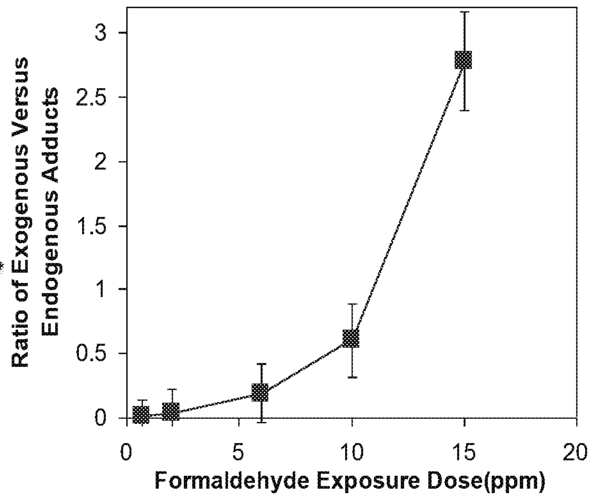
15 ppm Rat NE

Exposure (ppm)	Exogenous adducts/10^7 dG	Endogenous adducts/10^7 dG	n
0.7±0.2	0.039±0.019	3.62±1.33	3*
2.0±0.1	0.19±0.08	6.09±3.03	4**
5.8±0.5	1.04±0.24	5.51±1.06	4
9.1±2.2	2.03±0.43	3.41±0.46	5
15.2±2.1	11.15±3.01	4.24±0.92	5

*4-6 rats combined
** 2 rats combined

Ratio of Exogenous to Endogenous Adducts





Non-Human Primate Study

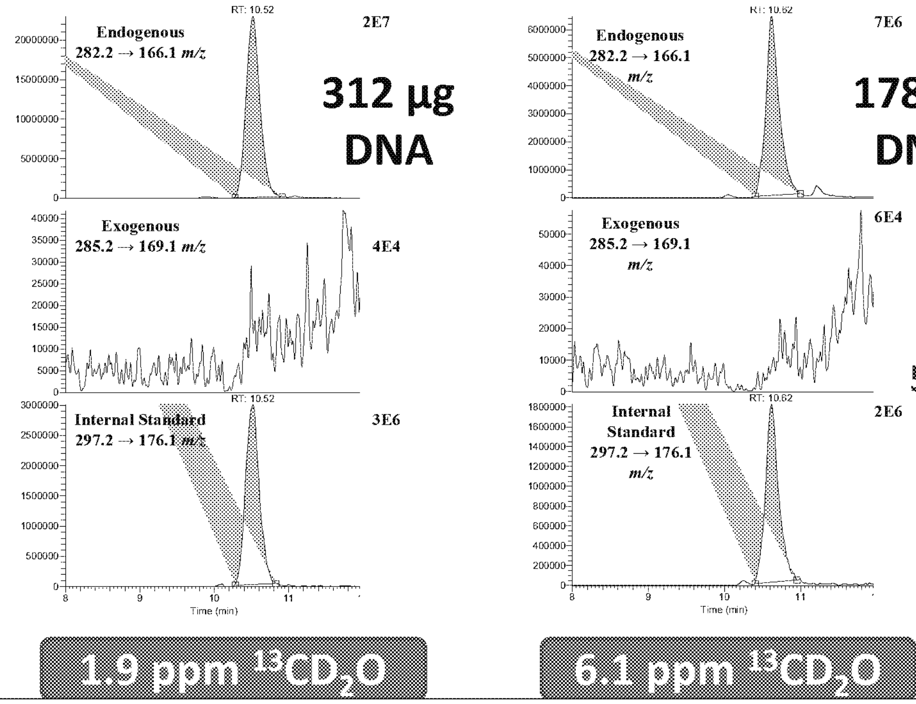
- $^{13}CD_2O$ Exposure for 2 days (6 hours/day) at 2 or 6 ppm (n=4)
- Cynomolgus Macaque
- Tissues (to date)
  - Nasal turbinates
  - Femoral Bone Marrow
  - Brain
  - Lung

Adduct Numbers in Primate Nasal Maxilloturinbates

Exposure concentration	Exogenous adducts/10^7 dG	Endogenous adducts/10^7 dG
1.9 ppm	0.25 ± 0.04	2.49 ± 0.39
6.1 ppm	0.41 ± 0.05	2.05 ± 0.53

n = 3 or 4

Primate Femoral Bone Marrow Endogenous and Exogenous Adducts



No Exogenous Adducts Detected with 5-10 fold >DNA

Note: We used ~20-30 ug for nasal tissue

On average we used between 20-30 ug of DNA for the Nasal tissues

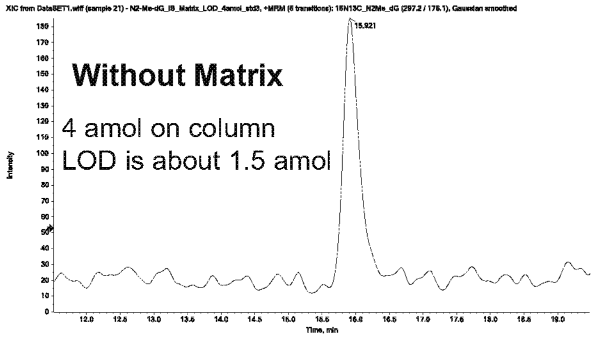
Adduct Numbers in Primate Bone Marrow

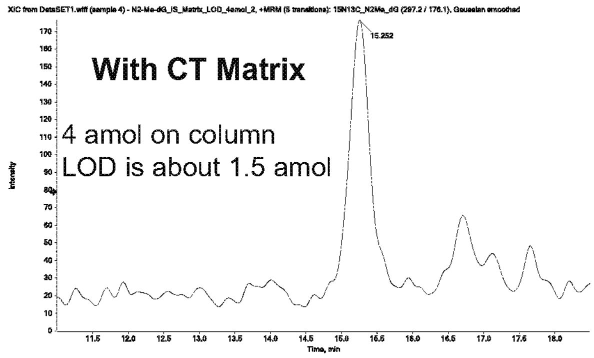
Exposure concentration	Exogenous adducts/10^7 dG	Endogenous adducts/10^7 dG
1.9 ppm	nd	17.48 ± 2.61
6.1 ppm	nd	12.45 ± 3.63

n = 4

Recent Improvements in Methodology

- Instrumentation
  SCIEX 6500 Triple Quadrupole MS
- LOD: 1.5 attomoles
- LOQ: 4 attomoles

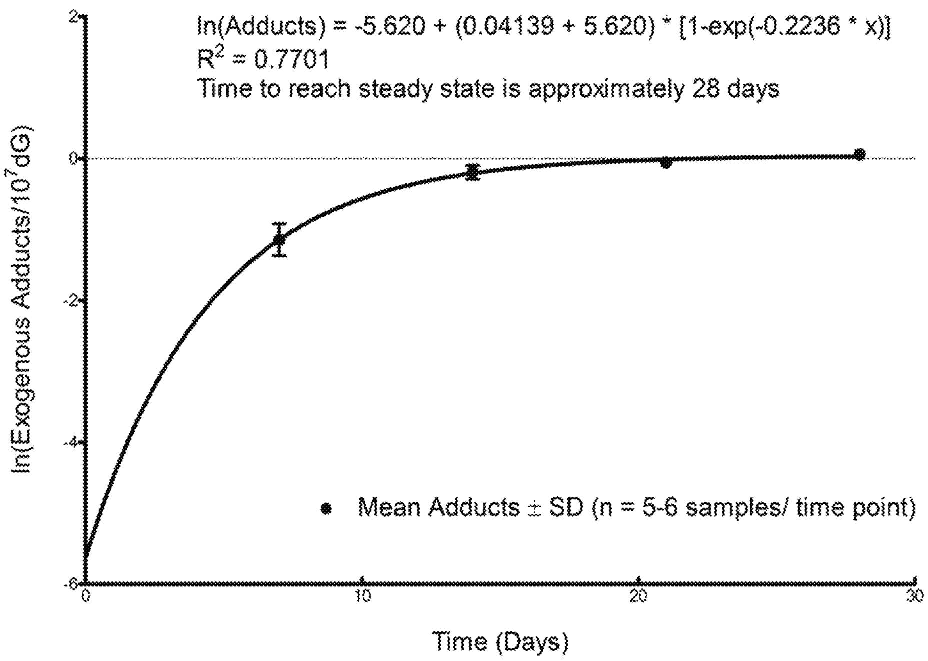




N^2-Methyl-dG Adducts in Rat Nasal Epithelium Following 2 ppm Exposure for up to 28 days (6 hr/day)

Time Points	Exogenous adducts/10^7 dG	Endogenous adducts/10^7 dG	n
7 day	0.35 ± 0.17	2.51 ± 0.63	5
14 day	0.84 ± 0.17	3.09 ± 0.98	5
21 day	0.95 ± 0.11	3.34 ± 1.06	5
28 day	1.07 ± 0.16	2.82 ± 0.76	5
28 day + 6 hr	0.85 ± 0.38	2.61 ± 0.55	5
28 day + 24 hr	0.83 ± 0.61	2.87 ± 0.65	5
28 day + 72 hr	0.64 ± 0.14	2.95 ± 0.71	5
28 day + 168 hr	0.76 ± 0.19	2.69 ± 0.45	6

Time to Steady-State for [$^{13}CD_2$]-HO-CH2-dG Adducts in Nasal Epithelium



N^2-Methyl-dG Adduct Numbers in Rat Bone Marrow Following 2 ppm Exposure for up to 28 days (6 hr/day)

Time Points	Exogenous adducts/10^7 dG	Endogenous adducts/10^7 dG	n
7 day	nd	3.37 ± 1.56	6
14 day	Nd	2.72 ± 1.36	6
21 day	nd	2.44 ± 0.96	6
28 day	nd[c]	4.06 ± 3.37	5
28 day + 6 hr	nd	2.41 ± 1.14	6
28 day + 24 hr	nd	4.67 ± 1.84	5
28 day + 72 hr	nd	5.55 ± 0.76	6
28 day + 168 hr	nd	2.78 ± 1.94	4

[c] One bone marrow DNA had 0.34 /10^7 dG exogenous N^2-HOMe-dG adducts in one bone marrow sample.

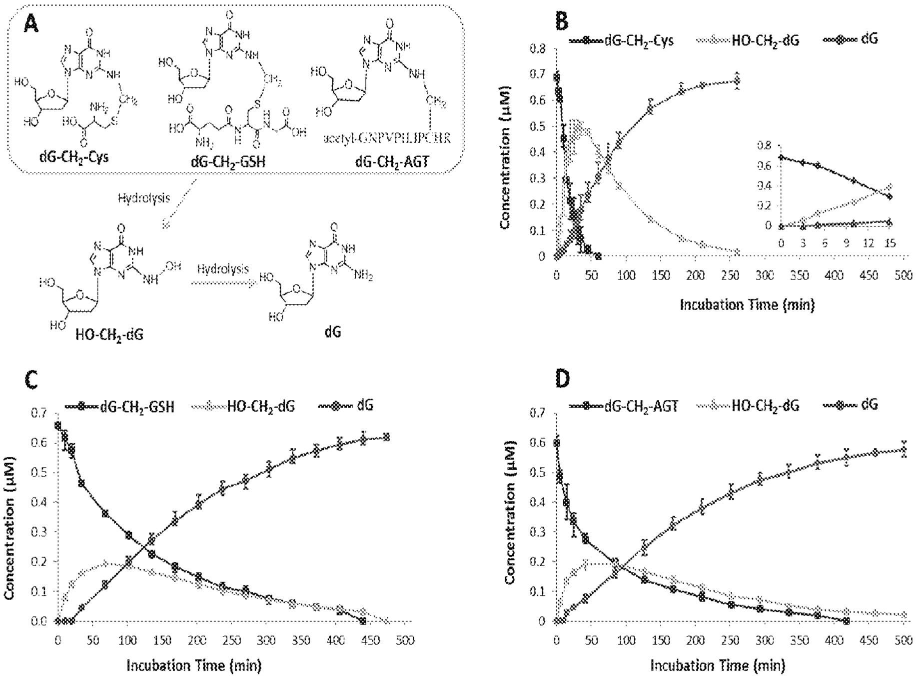
N^2-Methyl-dG Adduct Numbers in Rat WBC Following 2 ppm Exposure for up to 28 days (6 hr/day)

Time Points	Exogenous adducts/10^7 dG	Endogenous adducts/10^7 dG	n
7 day	nd	4.91 ± 3.71	4
14 day	nd	3.01 ± 0.54	4
21 day	nd	3.53 ± 0.72	4
28 day	nd	3.53 ± 0.72	4

Studies on Potential Artifact for Endogenous N^2-HOMe-dG Adducts

- The EPA asked us to rule out potential artifacts in our DNA isolation, reduction and hydrolysis. The amine group in Tris somehow interferes with DNA or nucleosides, and then forms N^2-HOMe-dG and artificially increases the detected amounts of endogenous DNA adducts.
- To address these issues, we compared 3 different batches of Tris•HCl buffer (BioXtra, BioUltra, BioPerformance) at the same concentration. Use of BioPerformance resulted in 10-fold greater numbers of N^2-HOMe-dG, but sodium phosphate buffer (BioXtra) had a peak area that was 100-fold lower than Tris•HCl buffer (BioPerformance). This was equal to approximately 35 amol N^2-Me-dG on column or 1.5 adducts/10^9 dG in 50 μg DNA, which was more than 180-fold lower than the average endogenous amounts of N^2-Me-dG in all tissues (2.71 ± 1.23 adducts/10^7 dG, n=205).
- The potential interferences present when sodium phosphate buffer was used were minimal, with less than 0.56% of the average endogenous amounts of N^2-Me-dG in all tissues.
- The average endogenous amount of N^2-HOMe-dG in all exposed tissues (n=397) was 2.82 ± 1.36 adducts/10^7 dG; and the average endogenous amount of N^2-HOMe-dG in all exposed tissues in the current 28 day study (n=158) was 2.78 ± 1.30 adducts/10^7 dG; while the average endogenous amount of N^2-HOMe-dG in all control tissues (n=47) was 2.47 ± 0.92 adducts/10^7 dG. These are not significantly different. Thus, it is clear that formaldehyde exposure does not increase endogenous N^2-HOMe-dG.

Spontaneous Hydrolysis of Formaldehyde DPCs Forms HO-CH_2-dG Adducts



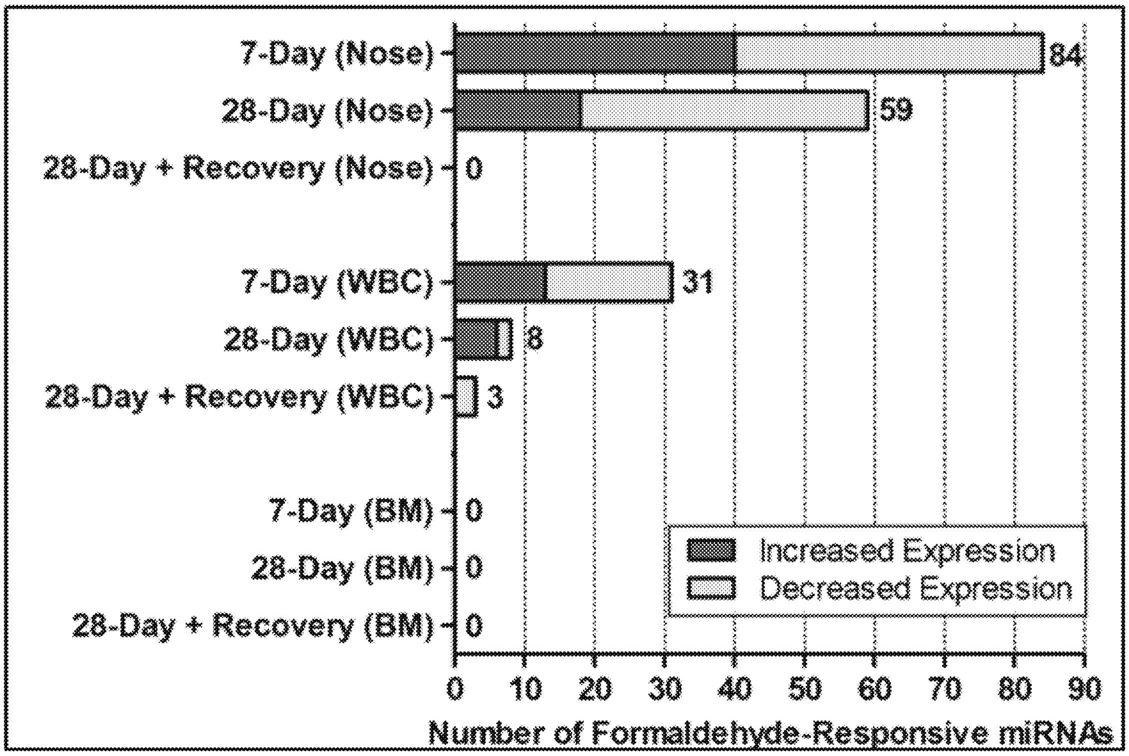
New Research Studies

- Epigenetic effects of inhaled formaldeyhde.
  - EHP paper for epigenetic studies in monkey maxilloturbinate.
  - 1 and 4 week exposures to 2 ppm formaldehyde and 1 week post exposure show changes in nasal tissue and WBC, but no changes in bone marrow. Different MiRNAs in different tissues and at different times.
- Development of hemoglobin adduct methods and data.
  - Ospina *et al* method was set up.
    - Exogenous adducts not found in exposed rat blood
    - Endogenous adducts are found
- Endogenous vs Exogenous N^6-formyllysine formation and hydrolysis.
  - Collaboration with MIT
  - Exogenous protein adducts only found in nasal epithelium and trachea
- Development of DNA-Protein Cross-link analysis
  - Spontaneous hydrolysis generates HO-CH_2-dG adducts
- Rat and primate comparisons of DPC and adducts vs IRIS human estimates.
- Additional rat and primate studies will examine ROS induced DNA adducts, formation of endogenous and exogenous DPCs, cytokine effects on epigenetic alterations, globin adducts and N^6-formyllysine.

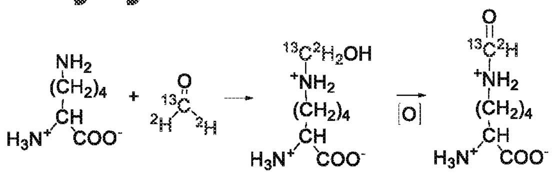
Nonhuman Primate Project

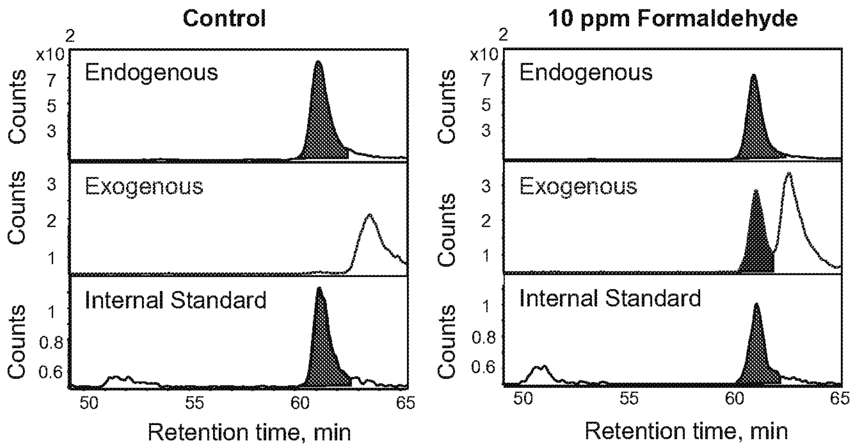
- Cynomolgus macaques were exposed to 0, 2, or 6 ppm $^{13}CD_2$ formaldehyde for 6 h/day for 2 days
- RNA samples were collected from the maxilloturbinate and hybridized to miRNA microarrays to compare genome-wide miRNA expression profiles of formaldehyde-exposed versus unexposed samples.
- 13 MicroRNAs had altered expression.
- Inhibition of apoptosis genes was predicted and demonstrated (Rager et al., 2013, EHP).

MiRNA Expression Profiles were Disrupted in the Rat Nose and WBC, but not the BM



Inhalation Exposure of Rats to [$^{13}CD_2$]-Formaldehyde leads to Formation of Labeled N^6-formyllysine in Nasal Tissue





Endogenous and Exogenous N^6-formyllysine Following a 6hr 9 ppm [$^{13}CD_2$]-Formaldehyde Exposure

N^6-Formylation per 10^4 Lys

Tissue	Nasal Epithelium		Lung		Liver		Bone Marrow	
Adduct type	**Endo**	**Exog**	**Endo**	**Exog**	**Endo**	**Exog**	**Endo**	**Exog**
Total Protein	2 ± 0.1	0.9 ± 0.1	3 ± 0.4	ND	3 ± 0.5	ND	4 ± 0.1	ND
Cytoplasmic	2 ± 0.4	0.8 ± 0.1	4 ± 0.6	ND	4 ± 0.1	ND	3 ± 0.3	ND
Membrane	2 ± 0.4	0.7 ± 0.2	3 ± 0.4	ND	3 ± 0.2	ND	2 ± 0.3	ND
Soluble nuclear	2 ± 1.0	0.5 ± 0.2	4 ± 0.3	ND	4 ± 0.7	ND	2 ± 0.2	ND
Chromatin bound	2 ± 0.4	0.2 ± 0.01	3 ± 0.2	ND	3 ± 0.3	ND	2 ± 0.1	ND

Edrissi et al., *Chemical Research in Toxicology:* DOI: 10.1021/tx400320u, October 2013.

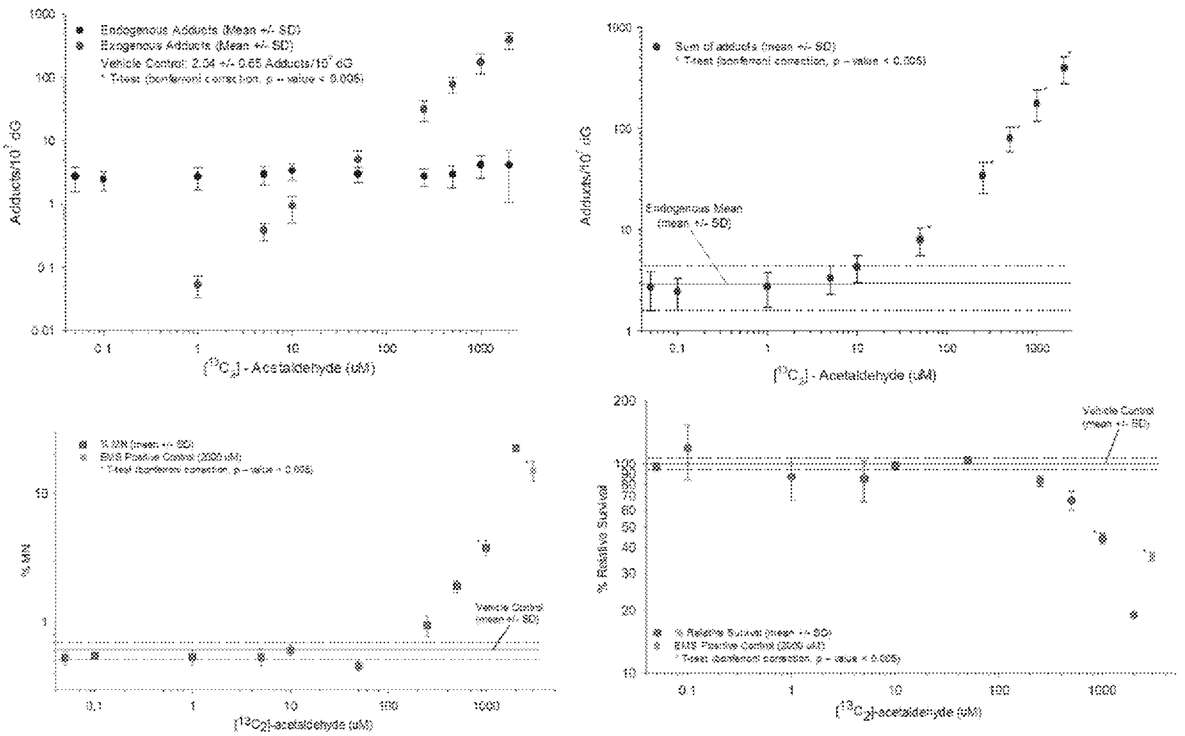
2 ppm 28 day Rat Study: % Exog/Endo N^6-Formyllysine

Exposure	7 d	14 d	21 d	28 d	28 d + 6 h post	28 d + 24 h post	28 d + 72 h post	28 d + 7 d post
Nasal Epithelium	19.8 ± 7.1	22.1 ± 12.7	24.8 ± 14.6	36.5 ± 15	22.8 ± 12.2	12.8 ± 4.8	13.2 ± 6.2	5.9 ± 1.0
Trachea	1.5 ± 0.5	1.2 ± 0.1	1.7 ± 0.9	1.4 ± 0.2	1.1 ± 0.1	1.2 ± 0.3	1.1 ± 0.3	0.8 ± 0.3
Lung	< 0.7	< 0.7	< 0.7	< 0.7	< 0.7	< 0.7	< 0.7	< 0.7
Liver	< 0.7	< 0.7	< 0.7	< 0.7	< 0.7	< 0.7	< 0.7	< 0.7
Bone Marrow	< 0.7	< 0.7	< 0.7	< 0.7	< 0.7	< 0.7	< 0.7	< 0.7

- Exogenous adducts were only detected in nasal epithelium and to a small extend in trachea
  - The exogenous adducts in distant tissues of lung, liver, and bone marrow did not increase beyond the natural isotope abundance level of ~0.7% for [M+2] ion of N^6-formyllysine
- Only nasal epithelium showed adduct accumulation over a 3-week period

Conclusions

- We have developed a series of highly specific and ultrasensitive methods that comprehensively demonstrate that inhaled formaldehyde does not reach distant tissues of rats and nonhuman primates.
- These methods utilize [$^{13}CD_2$]-formaldehyde for the exposures so that both endogenous and exogenous DNA, globin and N^6-formyllysine adducts can be distinguished and quantitated.
- The assays were conducted in two independent laboratories and have confirmed that [$^{13}CD_2$]-formaldehyde does not reach distant tissues such as blood and bone marrow.
- This research raises serious issues regarding the plausibility that inhaled formaldehyde causes leukemia. It seriously challenges the epidemiologic studies in several ways, including accurate exposure assessment, confounders and a lack of consistency across human and animal evaluations of carcinogenesis.



Moeller B C et al. Toxicol. Sci. 2013;toxsci.kft029

Collaborators and Sponsors

- Kun Lu
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- Tom Starr
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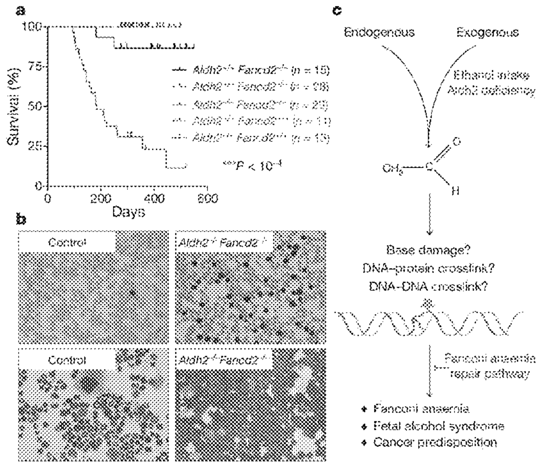
- NIEHS Center for Environmental Health and Susceptibility (P30 ES 10126)

Linearized Multistage Model for Cancer Risk Assessment

- The LMS model has been the default model for the EPA since 1986.
- It is highly public health conservative.
- Dr. Kenny Crump, the originator of the LMS model, has stated that this model
  - ***incorporates no biology, and***
  - ***will over estimate cancer risks by several orders of magnitude if nonlinear data are known***

Acute leukaemia in *Aldh2*$^{-/-}$ *Fancd2*$^{-/-}$ mice.



F Langevin *et al.* *Nature* **475**, 53-58 (2011) doi:10.1038/nature10192

nature

A Novel Bottom Up Approach to Bounding Potential Human Cancer Risks from Endogenous Chemicals

Thomas B. Starr, PhD
TBS Associates, Raleigh NC

SOT RASS Webinar
13 November 2013

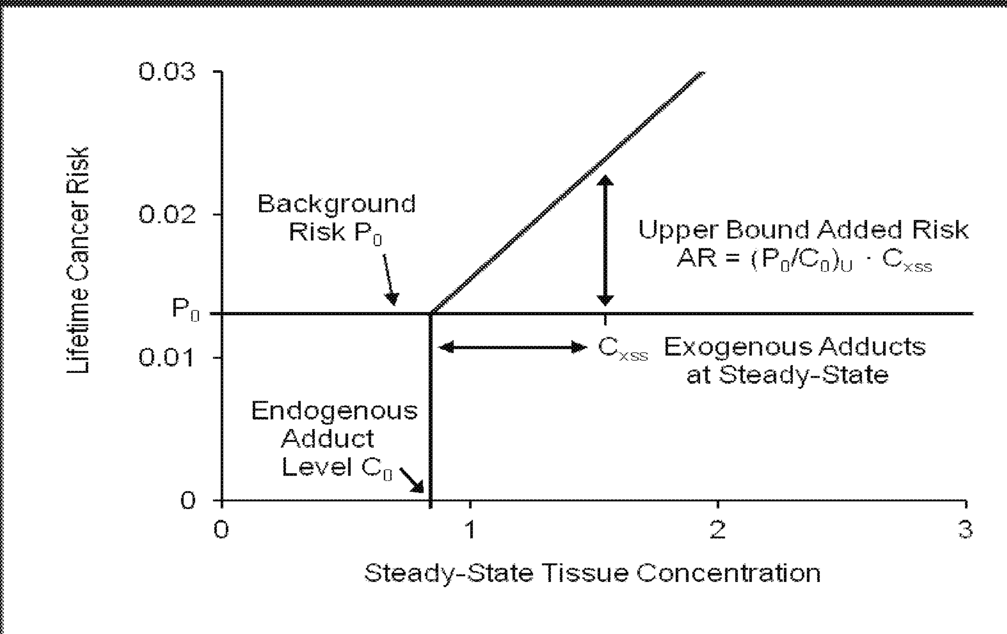
Typical Top Down Approach

- Cancer and exogenous exposure data extracted from epidemiology studies or laboratory animal bioassays
- Empirical or biologically-based dose-response models fit to cancer data vs exogenous exposure, e.g., airborne concentration, cumulative exposure
- Estimated $BMDL_x$ used to calculate upper bound unit risk for use in linear extrapolation or, alternatively, to compute MOEs for substances with nonlinear MOAs

The Bottom Up Approach

- Suitable for chemicals present in the body as a result of normal endogenous processes, e.g., metabolism
- Attributes all background risk P_0 to tissue-specific endogenous background exposure C_0
- Assumes linear dose-response for added risk AR vs exogenous exposure C_{xss}, with upper 95% confidence bound slope estimate $(P_0/C_0)_U$: $AR = (P_0/C_0)_U \cdot C_{xss}$
- P_0 from US SEER cancer statistics or bioassay data
  C_0 and C_{xss} data from short-term human/animal studies

Bottom Up Approach Elements



Bottom Up Approach Features

- Bounds low-dose cancer risk without using high dose cancer data from epidemiology studies or animal bioassays

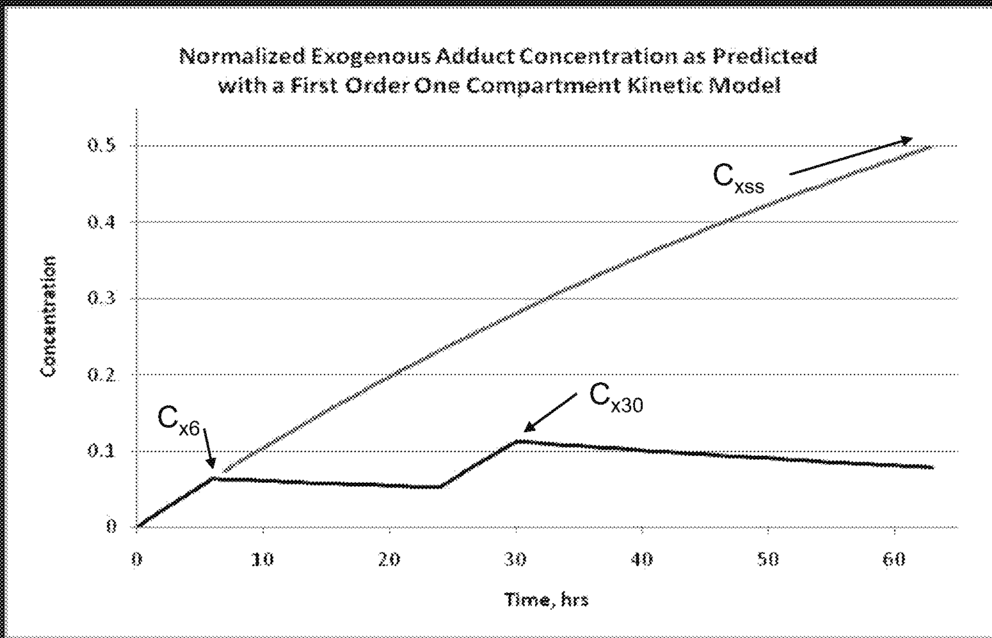
- Provides an independent "reality check" on extrapolations from high-dose data

- Conservative:
  - All background risk attributed to endogenous background exposure
  - Assumes linearity at low doses
  - Upper bound estimates of lifetime risk

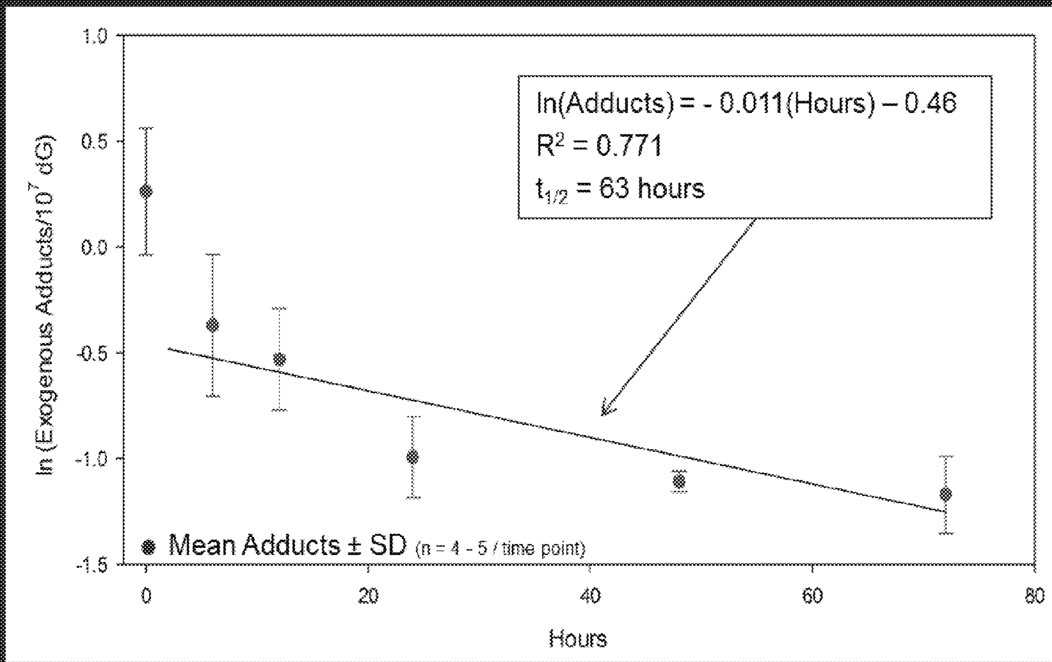
Estimating Steady-State Exogenous Adducts from Time Point-Specific Data

- Used one compartment model with constant forcing and first order elimination with half-life $T_{1/2} = T \cdot \ln(2)$
- For N^2-hydroxymethyl-dG adducts in rats (10 ppm for 6 hrs)
  $T_{1/2} = 63$ hrs , $T = 90.9$ hrs (Swenberg 2012)
- At the end of one 6 hour exposure:
  $C_{xss} = C_{x6} / (1 - \exp(-6/T)) = 15.65 \cdot C_{x6}$
- After two 6 hour exposures on consecutive days:
  $C_{xss} = C_{x30} / \{[1 - \exp(-6/T)] \cdot [1 + \exp(-24/T)]\} = 8.85 \cdot C_{x30}$

One Compartment Model: Adduct Time Profile

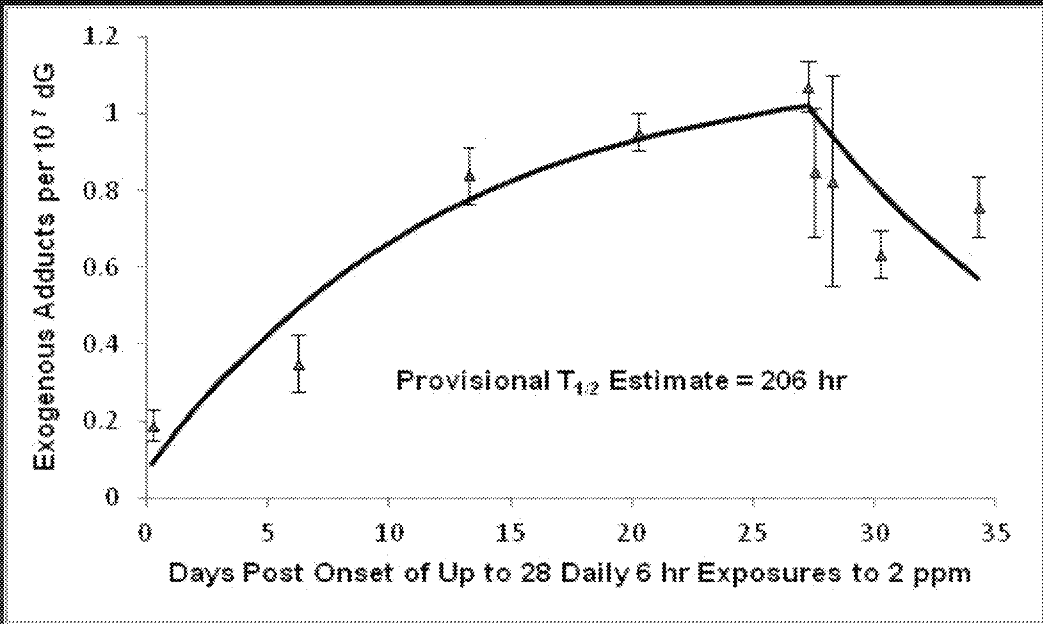


N^2-Hydroxymethyl-dG Elimination Half-Life Data



One 6 hr exposure of rats to 10 ppm, Swenberg et al., 2012

New N^2-Hydroxymethyl-dG Elimination Half-Life Data



Swenberg (unpublished data)

N^2-hydroxymethyl-dG Adducts in Monkeys Exposed Twice for 6 hrs to 2 ppm $^{13}CD_2O$

Tissue	Endogenous Adducts at 30 hrs	Exogenous Adducts at 30 hrs	Exogenous Adducts at Steady-State
Nasal Epithelium Mean ± se Lower 95% Bound	2.49 ± 0.23 2.11 C_{0L}	0.25 ± 0.020 C_{x30}	2.21 ± 0.18 C_{xss}
Bone Marrow Mean ± se Lower 95% Bound	17.5 ± 1.31 15.34 C_{0L}	< 0.00103[a] C_{x30}	<0.00912[a] C_{xss}

a: no exogenous adducts were detected in bone marrow; upper limit estimate based on the detection limit reported in Moeller et al. (2011).

Comparison of Bottom Up and Top Down Upper Bound Added Risk Estimates

Cancer	Background Risk P_0	Bottom-Up Slope P_0/C_{0L}[a]	C_{xss} at 2 ppm	Bottom-Up Risk at 1 ppm[b]	USEPA Risk at 1 ppm
NPC	7.25×10^{-4}	3.44×10^{-4}	2.21 ± 0.18	0.038×10^{-2}	1.1×10^{-2}
LEU	1.30×10^{-2}	8.50×10^{-4}	< 0.00912	$< 3.9 \times 10^{-6}$	5.7×10^{-2}

For NPC, $AR_{BU} = (3.44 \times 10^{-4} \cdot 2.21) / 2 = 0.038 \times 10^{-2}$

$= 29.8$-fold lower than AR_{EPA}

For LEU, $AR_{BU} = (< 8.5 \times 10^{-4} \cdot 0.00912) / 2 = < 3.9 \times 10^{-6}$

$= > 14{,}615$-fold lower than AR_{EPA}

Bottom Up Uncertainties (Human Analysis)

- P_0 very precise due to large number of cases in US population of more than 300,000,000:
  Annually, > 2,550 NPC, > 45,880 LEU

  NPC $P_0 = 7.2500 \times 10^{-4}$, $P_{0U} = 7.2656 \times 10^{-4}$
  LEU $P_0 = 1.3000 \times 10^{-2}$, $P_{0U} = 1.3011 \times 10^{-2}$

- C_0 uncertain due to small monkey sample sizes:
  Nasal $C_0 = 2.49 \pm 0.23$, $C_{0L} = 2.11$
  Bone Marrow $C_0 = 17.5 \pm 1.31$, $C_{0L} = 15.34$

- $T_{1/2}$ and C_{xss} uncertain due to small rat sample sizes

Top Down Uncertainties (Human Analysis)

NPC: - VERY small number of deaths: 2 UnExp, 7 Exp
- coarsely stratified cumulative exposure metric
- marginally significant trend due to excess in highest exposure category (3 deaths)
- non-monotonic dose-response

LEU: - small number of deaths: 7 UnExp, 116 Exp
- coarsely stratified cumulative exposure metric
- non-significant positive trend due largely to ~ 47% deficit in Unexposed group relative to the Exposed groups
- no dose-response in Exposed groups

Generalizing to Other Chemicals

- Methanol (metabolized to formaldehyde)
- Acetaldehyde (N^2-hydroxyethyl-dG adducts
- Vinyl Acetate (metabolized to acetaldehyde)
- Vinyl Chloride (metabolized to chloroethylene oxide, producing 1 oxoethyl and 3 exocyclic etheno adducts)
- Ethylene Oxide (4 hydroxy-ethyl adducts)

Some Criteria for Use in Risk Assessment

- Specific target sites in humans (epidemiology studies)
- Valid biomarkers of target site exposure that are plausibly correlated with the apical endpoint
- High precision/accuracy measurements that distinguish between endogenous / exogenous sources at low exogenous exposure levels
- Use conservative assumptions to fill data gaps
- Use to "reality check" and, when appropriate, replace top down analyses

Advantages of the Bottom Up Approach

- Uses background cancer risk in humans
- Uses background (endogenous) adduct concentrations
  in humans, if available, or short-term animal data
  and equivalence assumptions
- Conservative:
  Linear at low doses (consistent with additivity)
  All background risk attributed to endogenous adducts
  Provides an upper bound on low-dose slope
- Produces a completely independent "reality" check
  on risk extrapolations from high-dose data

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Methanol (Noncancer) Assessment: Accounting for Background Blood Levels

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National Center for Environmental Assessment
November 17, 2013

Disclaimer: The views expressed in this paper are those of the authors and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency.

History

Date	Event	Result
1988	Original IRIS posting	RfD for ↓ brain wt. in 90-d oral rat study
2003	EPA Scoping meetings	Sufficient rodent inhalation bioassays; Insufficient human studies; Need rodent/monkey/human PBPK models; Focus on developmental risk from exogenous exposures
2006	PBPK models developed	Predict internal doses from exogenous exposures
2006-	Agency and Interagency Reviews	Should PBPK models include a background term? Should EPA base RfD/C on exogenous exposures?
2011	Public and External Reviews	Background term added to models. RfD ↑ 5-fold; RfC ↑ 10-fold. RfD/C based on exogenous exp., but relationship to background blood levels discussed.
2013	Public and Follow-up EPR	Endogenous background methanol blood level assumption clarified.

Key Determinations

- Critical effects
- Appropriate moiety and internal dose metric for analyzing the critical effects
- Lowest internal dose increase over endogenous background associated with a risk that can be reliably estimated (PBPK/BMD analysis)
- Internal dose increase that is not likely to cause an appreciable health risk (Uncertainty Factors)
- Derivation of RfD/C from internal dose (human PBPK)
- Relation of RfD/C to endogenous background

Critical Effects

Human adults:	Acute exposures: death; vision/CNS; slight neuro & immune effects
Monkey neonates:	Uncertain dose-response: short gestation, VDR
Monkey adults:	Limited study: liver, heart, renal & brain effects
Rodent fetuses:	High quality studies in mice: extra cervical ribs, cleft palate, exencephaly, reduced fetal wt & pup survival, ossification delay
Rodent neonates:	Extensive studies with limited documentation: reduced weight of brain, pituitary, and thymus
Rodent adults:	Well documented rat and mouse studies, marginal effects

Proximate Moiety

Possible MOAs: Methanol, formaldehyde, formate, ROS

Key considerations:

- Methanol - metabolized to formaldehyde at multiple organ sites
- Formaldehyde – reactivity limits transport as free formaldehyde
- Formate - blood levels not correlated with developmental toxicity
- ROS – conflicting evidence; induced by methanol

EPA assumptions:

- PBPK models accounts for species metabolic differences
- PBPK model of parent methanol adequate for critical effects
- All MOAs require methanol to be present at the target site

Internal Dose Metric

- For BMD analysis, the dose metric should be as closely related to the health effect under consideration as possible
- Mouse cervical rib - Peak (C_{max}) methanol in blood (mg/L)
  - Exposure magnitude more important than duration
  - Short gestational window of susceptibility (GD 7-8)
  - Improves dose-response model fit
- Rat brain weight - AUC methanol in blood (mg-hr/L)
  - Duration is a factor in developmental and subchronic studies
    - Effect increases with duration (e.g., gestational + neonatal > gestational)
    - Effect observed following 90 day subchronic exposure
  - Improves dose-response model fit

Increase Over Background

GD6 blood levels (C_{max} over background); inhalation exposure
Rogers et al. (1993) Mouse Study

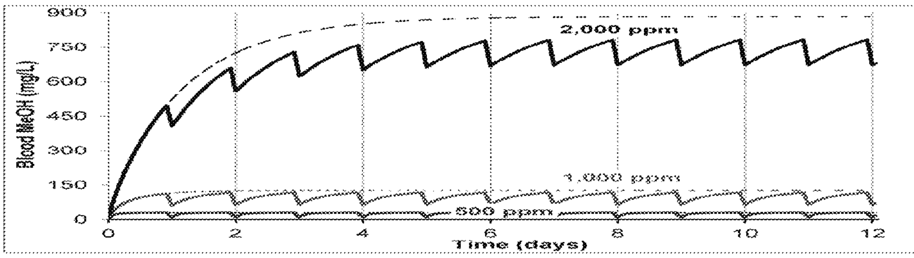
Exposure (ppm)	C_{max} - C_{bg} (mg/L)[a]	Cervical Rib/Litter (%)
0	**0**	28
1,000	**61.4**	33.6
2,000	**485.4**	49.6
5,000	**2,124.4**	74.4

[a]Reported C_{max} background levels of 1.6 mg/L were subtracted from reported C_{max} values.

Increase Over Background

Simulated C_{max} and AUC over Background; 22 h/day inhalation

NEDO (1987) developmental study of Sprague-Dawley rats



Exposure concentration (ppm)	C_{max} (mg/L)	$C_{max} - C_{bg}$ (mg/L)	AUC ($C - C_{bg}$) (mg-hr/L)
500	28.7	25.7	**547**
1,000	118	115	**2,310**
2,000	783	780	**17,500**

Increase Over Background

Analyzing Increase Over Background vs Critical Effects

BMD Modeling Results

	Rogers et al. (1993b) mouse inhalation developmental study	NEDO (1987) rat inhalation developmental study
	5% increase in incidence of extra cervical rib (C_{max})	1 SD reduction in brain weight (AUC)
BMDL = $POD_{internal}$	43.1 mg/L	858 mg-hr/L

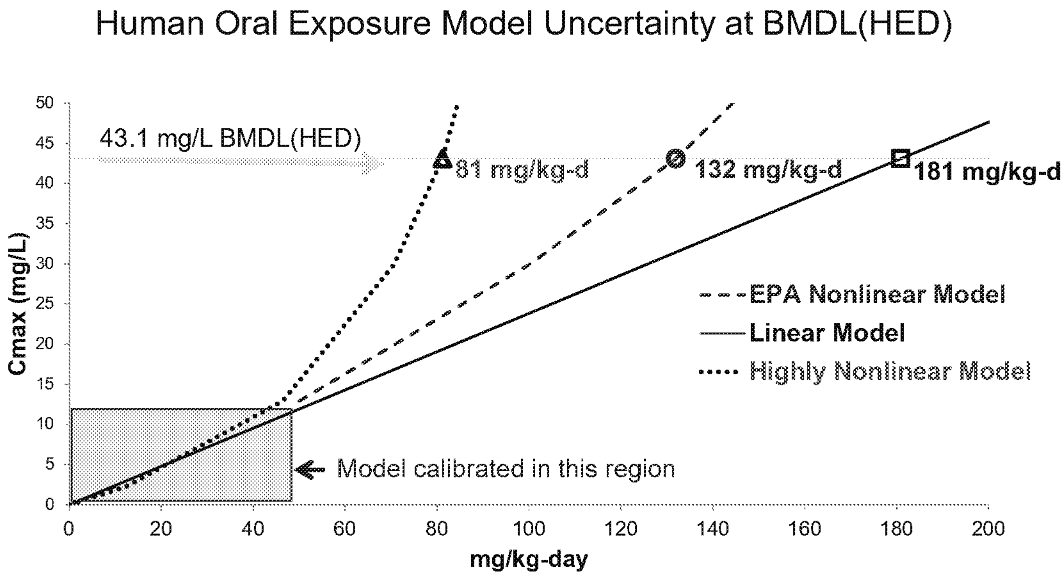
Uncertainty Factors

Estimating the Internal Dose Above Background That Would Not Cause Appreciable Health Risk

	Rogers et al. (1993b) mouse inhalation developmental study	NEDO (1987) rat inhalation developmental study
	5% increase in incidence of extra cervical rib (C_{max})	1 SD reduction in brain weight (AUC)
BMDL = $POD_{internal}$	43.1 mg/L	858 mg-hr/L
$POD_{internal}$/UFs*	0.43 mg/L	8.58 mg-hr/L

* UF_A =3; UF_D = 3; UF_H = 10; UF_S = 1; UF_L = 1; product of all UFs = 100

UFs Applied to Internal PODs



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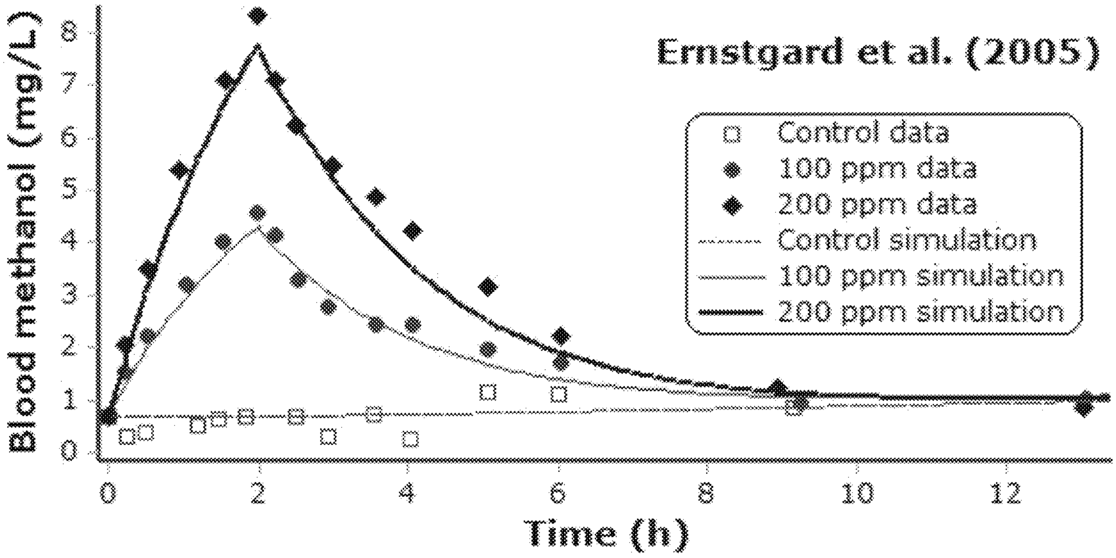
Human PBPK Model Validation



RfD/C via Human PBPK Model

Human PBPK Model Assumptions

- Endogenous background = 2.54 mg/L
  - Zero-order rate from stomach tuned to yield this level
- Metabolism assumed to be saturable
  - Data were sufficient to identify a Km
  - Slight nonlinearity in the range of interest
- Adult non-pregnant female
- Continuous inhalation exposure
- Idealized oral ingestion pattern (percents of daily dose):
  - 25% at 7 a.m., 10% at 10 a.m., 25% at 12 p.m., 10% at 3p.m., 25% at 6 p.m., and 5% at 9 p.m.
  - Simulations run to “periodicity”, then AUC and Cmax calculated

RfD/C Derivation

	Rogers et al. (1993b) mouse inhalation developmental study	NEDO (1987) rat inhalation developmental study
	5% increase in incidence of extra cervical rib (C_{max})	1 SD reduction in brain weight (AUC)
BMDL = $POD_{internal}$	43.1 mg/L	858 mg-hr/L
$POD_{internal}$/UFs	0.43 mg/L	8.58 mg-hr/L
RfC (mg/m3)*	20.0	17.8
RfD (mg/kg/day)*	1.9	5.2

* Exposure predicted to yield a blood concentration equal to $POD_{internal}$/UFs using the human PBPK with a background blood concentration of 2.5 mg/L.

Methanol Background in Blood

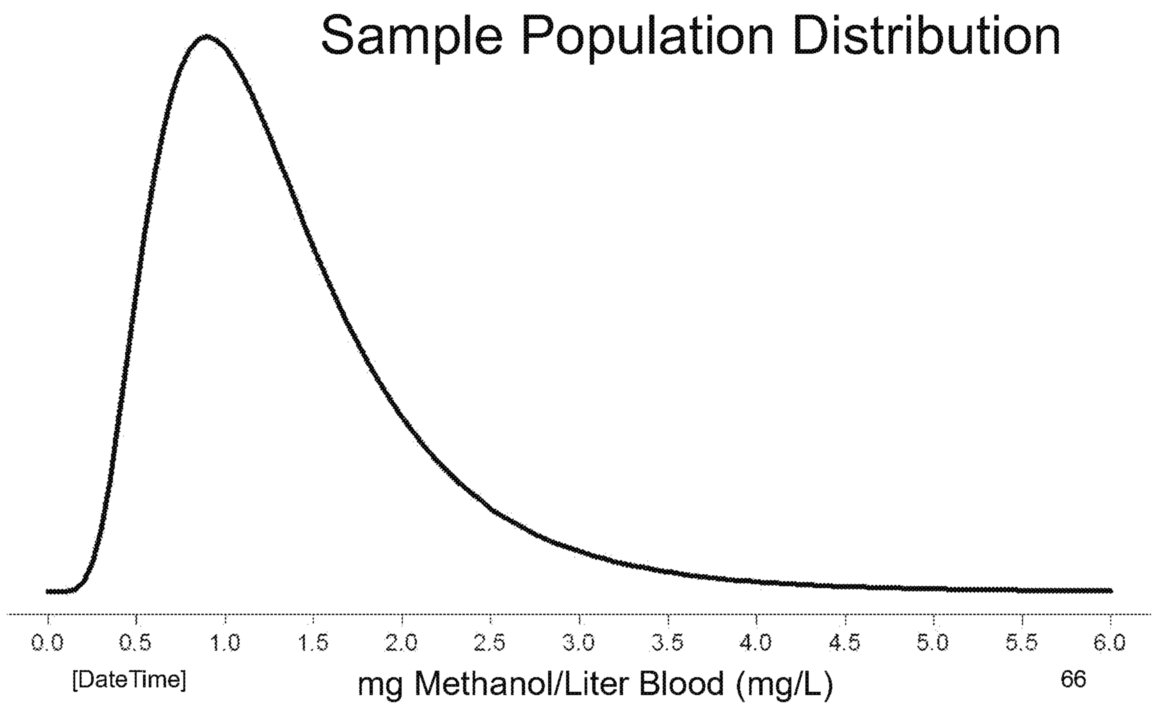
Six Human MeOH Studies With No Substantial Dietary Restrictions

Description of human subjects	Methanol (mg/L) mean ± SD[a] (Range)	Reference
12 adults who drank no alcohol for 24 hr	1.7 ± 0.9 (0.4-4.7)	Batterman and Franzblau (1997)
12 adults who drank no alcohol for 24 hr	1.8 ± 0.7 (No range data)	Batterman et al. (1998)
3 males who ate a breakfast with no aspartame-containing cereals and no juice	1.82 ± 1.21 (0.57-3.57)	Lee et al. (1992)
5 males who ate a breakfast with no aspartame and no juice (2nd experiment)	1.93 ± 0.93 (0.54-3.15)	
35 adults - no alcohol for 1 wk, fasted 4 hrs	0.64 ± 0.45	Sarkola & Eriksson (2001)
30 adults. No diet restrictions. Blood levels estimated from concentrations in breath.	1.25 ± 0.29[a] (0.45-1.7)	Turner et al.(2006)
18 males, fasted 3 hr, no other diet restrictions	2.62 ± 1.33 (0.7-5.2)	Woo et al. (2005)

Methanol Background in Blood

- Six studies that did not involve substantial dietary restrictions were used to fit a lognormal distribution.
- Weighted by ability to represent U.S. population.
  - *Sarkola and Eriksson* (2001) restricted alcohol consumption so was given a 0.48 weight, commensurate with percent of U.S. population that are not regular drinkers (CDC, 2011).
  - *Woo et al.* (2005) used an Asian population that has variants of the gene coding for alcohol dehydrogenase so was given a 0.036 weight, commensurate with the Asian fraction of the U.S. population (SSDAN CensusScope, 2010).

Methanol Background in Blood



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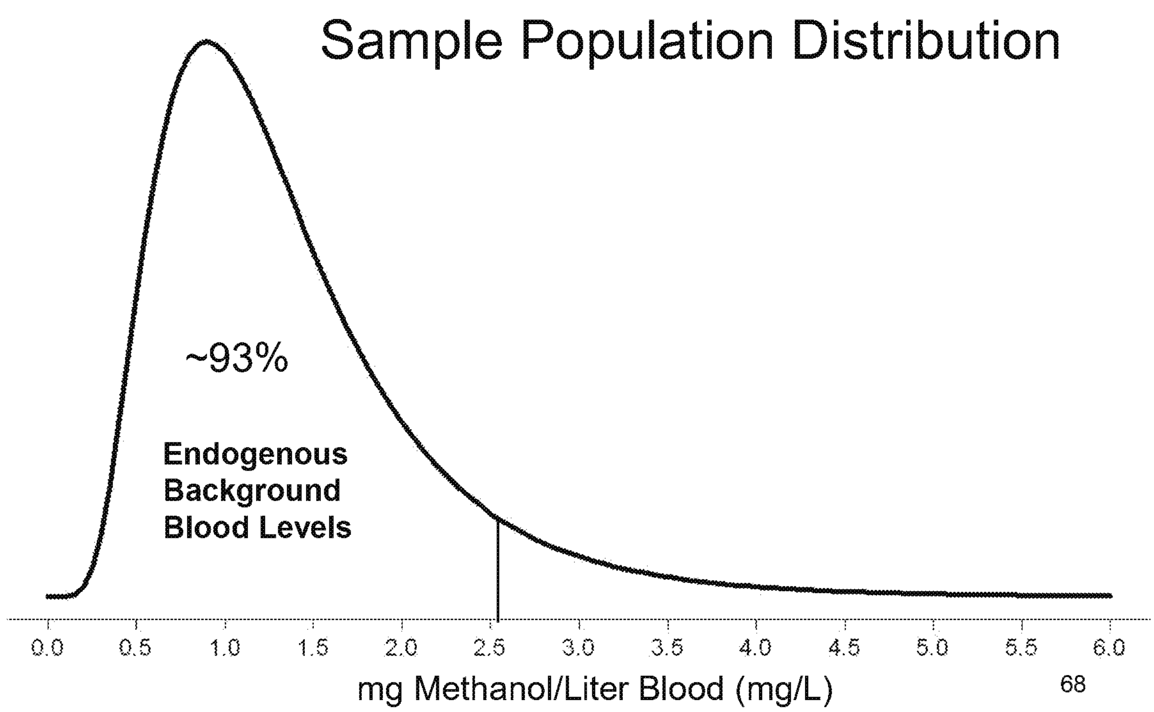
Endogenous Background

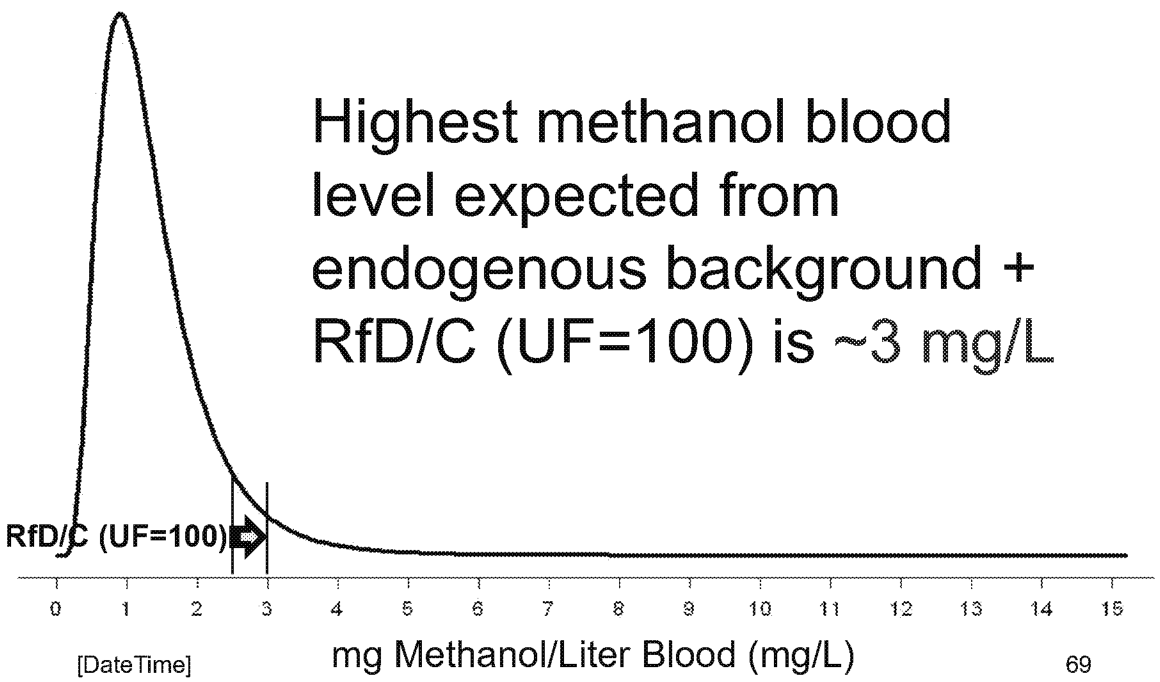
- RfD/C is an exogenous exposure that adds to endogenous background (metabolism + ordinary diet)
- The U.K. estimates an upper bound of endogenous methanol background of 23 mg/kg-day.
- EPA PBPK model predicts 23 mg/kg-day would result in methanol blood level of 2.54 mg/L
- EPA assumes ~2.5 mg/L is upper end of endogenous methanol background in blood

Endogenous Background



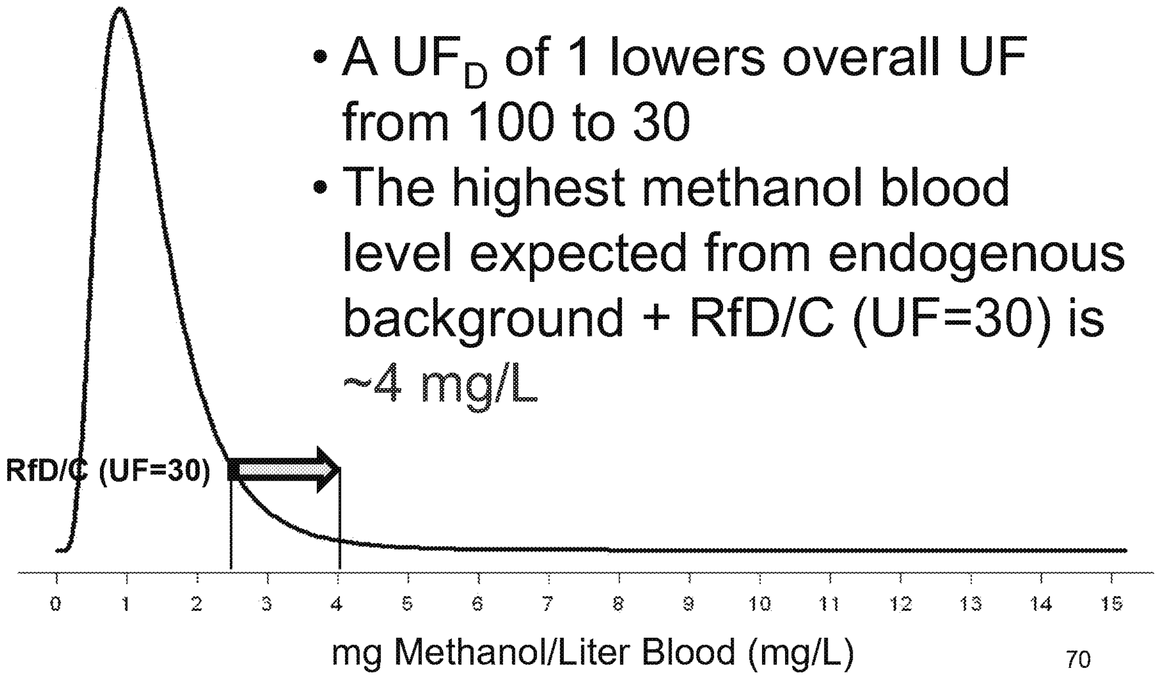
Endogenous Background + RfC/D

Highest methanol blood level expected from endogenous background + RfD/C (UF=100) is ~3 mg/L



Database Uncertainty Factor

- A UF_D of 1 lowers overall UF from 100 to 30
- The highest methanol blood level expected from endogenous background + RfD/C (UF=30) is ~4 mg/L



RfD Definition

"The RfD (expressed in units of milligrams per kilogram per day [mg/kg-day]) is defined as an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (**<u>including sensitive subgroups</u>**) that is likely to be **<u>without an appreciable risk</u>** of deleterious effects during a lifetime."

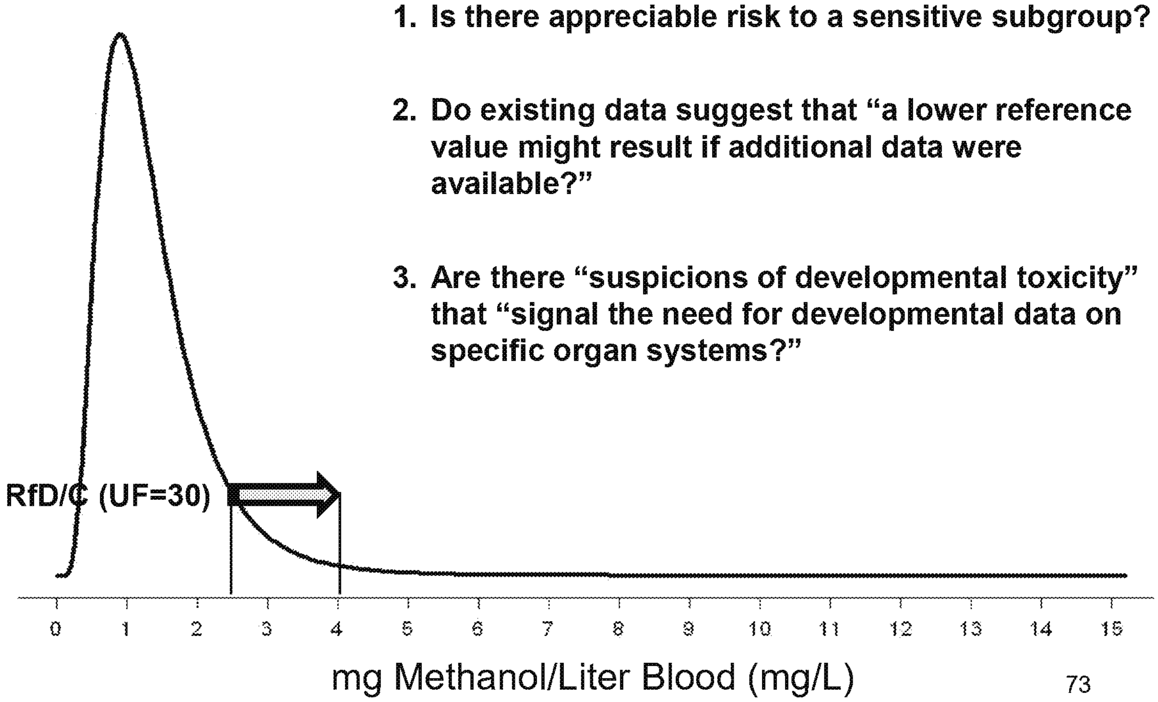
EPA UF_D Guidance

"In addition to identifying toxicity information that is lacking, review of existing data may also suggest that a lower reference value might result if additional data were available."

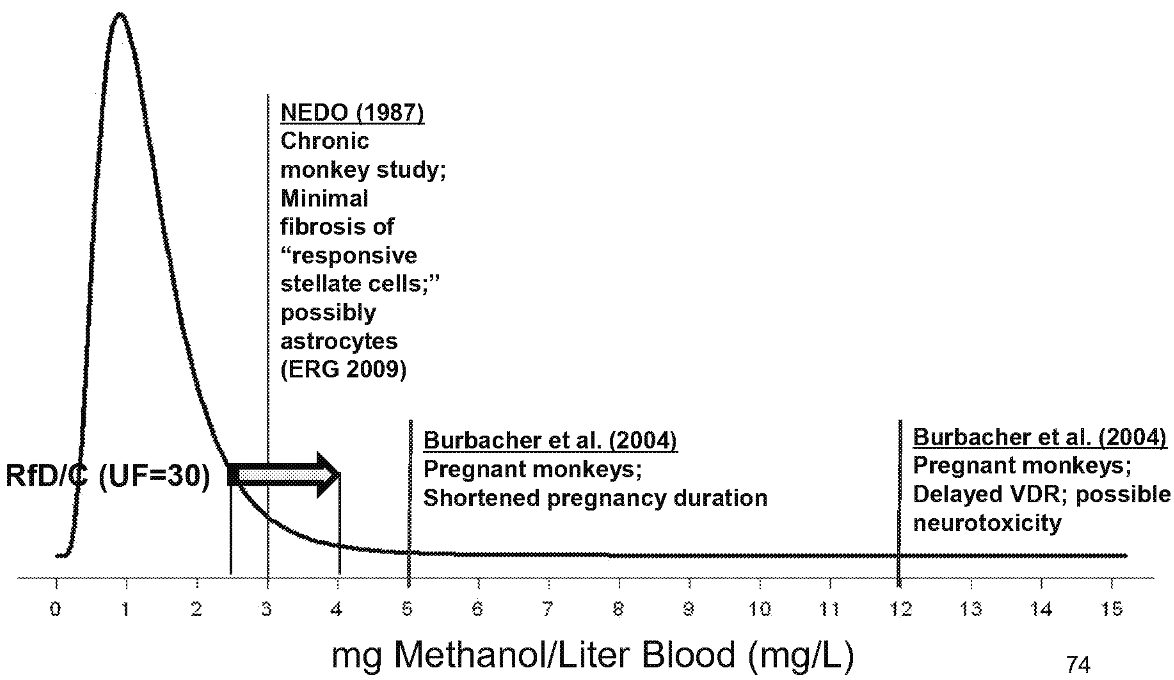
"If data from the available toxicology studies raise suspicions of developmental toxicity and signal the need for developmental data on specific organ systems (e.g., detailed nervous system, immune system, carcinogenesis, or endocrine system), then the database factor should take into account…"

Database Uncertainty Factor

1. Is there appreciable risk to a sensitive subgroup?
2. Do existing data suggest that "a lower reference value might result if additional data were available?"
3. Are there "suspicions of developmental toxicity" that "signal the need for developmental data on specific organ systems?"



Database Uncertainty Factor



Database Uncertainty Factor

1. **Is there appreciable risk to a sensitive subgroup?**

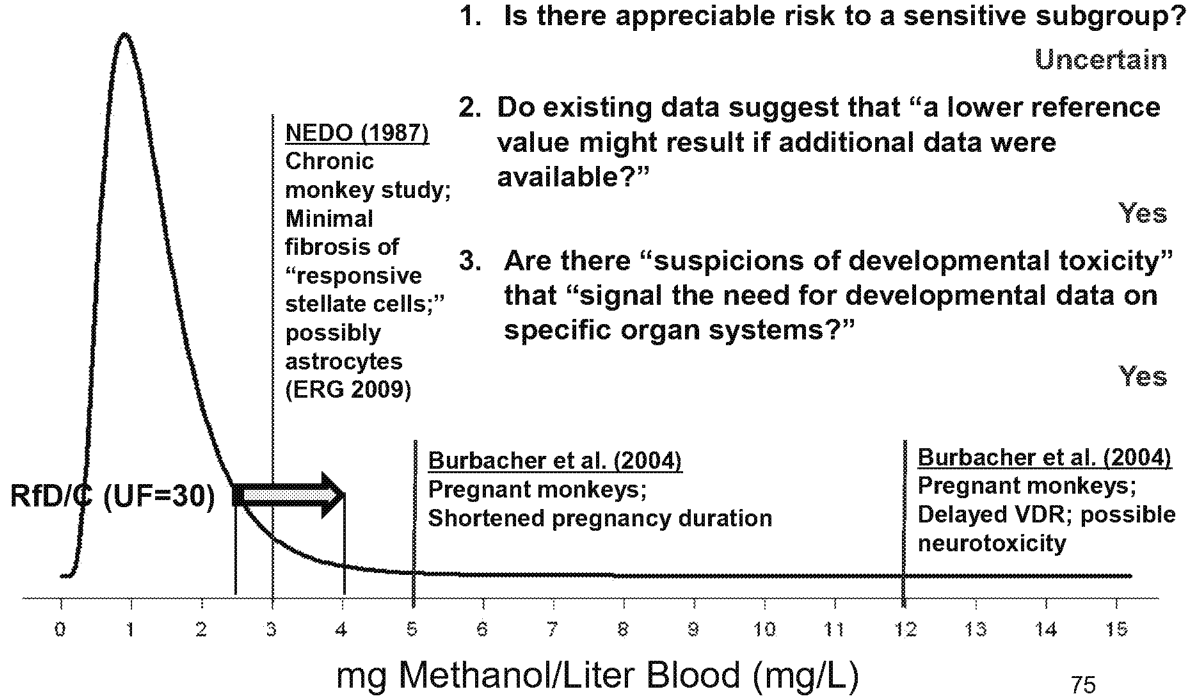
   Uncertain

2. **Do existing data suggest that "a lower reference value might result if additional data were available?"**

   Yes

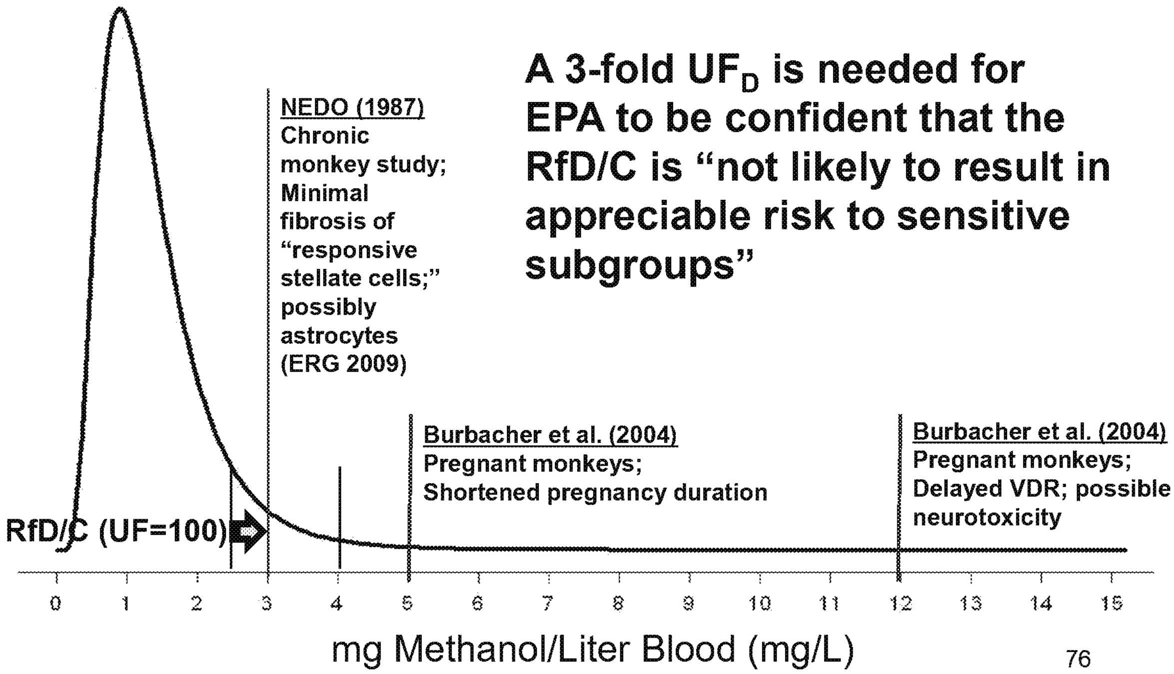
3. **Are there "suspicions of developmental toxicity" that "signal the need for developmental data on specific organ systems?"**

   Yes



A 3-Fold UF_D (UF=100) is Justified

A 3-fold UF_D is needed for EPA to be confident that the RfD/C is "not likely to result in appreciable risk to sensitive subgroups"



Summary

Critical effects: Developmental; extra cervical rib in mice, reduced brain weight in rats

Moiety: Parent methanol

Metric: Blood C_{max} for cervical rib; Blood AUC for brain weight

PBPK/BMD analysis: Estimated BMDLs from blood C_{max} and blood AUC doses with endogenous background subtracted

Uncertainty Factors: UF_A of 3 + UF_D of 3 + UF_H of 10 = 100; Applied to blood POD to avoid extrapolating beyond human PBPK model calibration range

RfD/C derivation: From blood methanol POD/100 using human nonpregnant PBPK model, assuming 2.5 mg/L endogenous background, saturable metabolism, continuous inhalation and idealized oral ingestion pattern

Relation to endogenous background: RfD/C is an exogenous exposure that adds to endogenous background (metabolism + ordinary diet)

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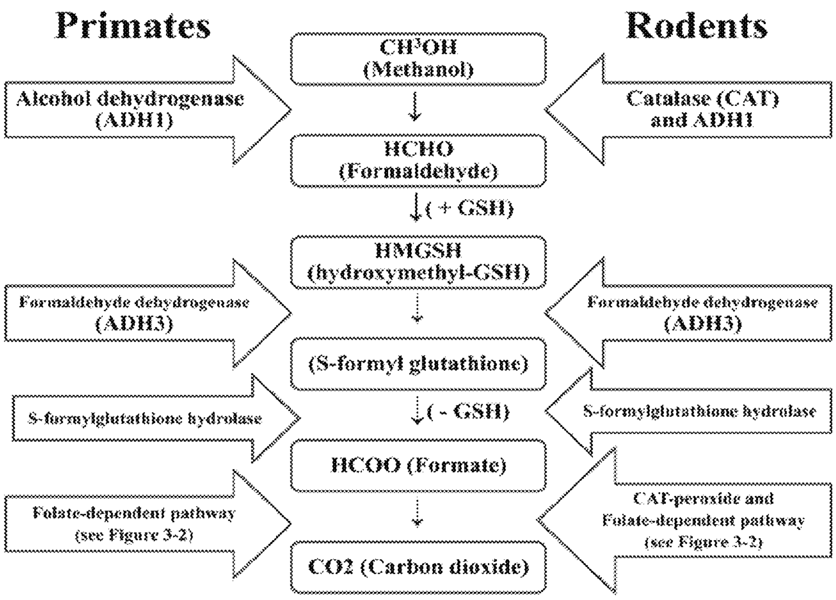
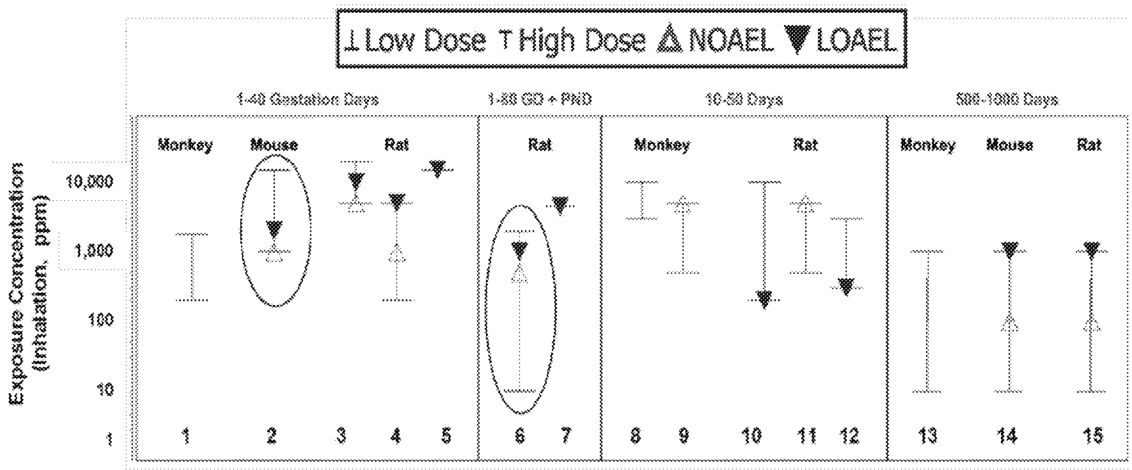
Extra Slide - Metabolism



Extra Slide - Methanol Effects



1. Shortened gestation
2-4. Skeletal/Reproductive
5. Pup weight
6. Pup brain weight
7. Cognitive deficits
8. CNS, CWM edema
10. Testosterone levels
12. Thyroid follicles
13. Liver-fibrosis Brain-Stellate cells
14-15. Minor effects